Paragraphs from current application Description of Preferred Embodiments

The degree of enhancement can be controlled by varying the ratio of thio-phosphate to phosphate used and/or by varying the time point of the addition of the modified phosphate to the fermentation reaction. The optimal ratio of thio-phosphate to normal phosphate that enhances protein synthesis of a given gene product must be determined for a given organism. The optimal ratio may also vary under different cellular physiological states such as that imparted by the vector system employed (i.e. plasmid or phage) or growth phase (trophophase versus idiophase). While a generally optimal ratio for most genes may emerge for a given organism it is still possible that an unusually unstable mRNA may be preferentially induced at lower ratios of thio-phosphate to phosphate levels. If the organism can tolerate an increase in total cellular protein than even stable mRNAs may be enhanced over time.

If the product is synthesized during the growth or the trophic phase then there are a number of possible points which may be rate limiting such as glucose uptake, glycolysis (often very fast), Kreb's cycle, enzymatic catalysis or product feedback inhibition. Thus the rate of fermentation, duration, and/or yield of product may be increased depending on which step is rate limiting. If the product is produced during the idiophase as a secondary metabolite then mRNA stabilization of catalytic enzymes involved in the synthesis of the metabolite may also enhance yields during this later stage of fermentation.

Thio-phosphate can also be used to increase the protein synthesis of many mRNAs. Generally an optimal ratio of thio-phosphate to phosphate must be found. Very high ratios of thio-phosphate to phosphate will result in a decrease in total protein synthesis. Optimal protein synthesis occurs at lower ratios where total cellular protein levels are not affected but total secreted protein levels may be enhanced. The ratio of thio-phosphate to phosphate rather than the total thio-phosphate level is important for conferring the degree of stabilization. The degree of enhancement may depend somewhat on the natural stability of the mRNA. Less stable and heterologous gene products may be enhanced preferentially. In yeast it is known that many mRNAs for extracellular hydrolytic enzymes such as amylase are atypically stable with half-lives more than ten hrs. In contrast, most yeast mRNAs have half-lives on the order of 20 mins. Very low ratios of thio-phosphate to phosphate (10%) in yeast appear to preferentially enhance the accumulation of the more typical mRNAs relative to the abundant and stable hydrolytic mRNA population. The degree of protein enhancement depends somewhat on the relative stability of corresponding mRNAs and can be as much as ten fold for less stable or heterologous gene products in bacteria and/or yeast. Note however, that the coordinated enhancement of many genes may increase the efficiency of specific processes in protein production such as the secretion of proteins above and beyond what is achieved through mRNA stabilization alone.

9/4/08

In the United States Patent and Trademark Office

Appn. Filed: 12/05/2001
Appn. #: 10/679,305
Applicant: Elizabeth Gay Frayne
Title: "Manipulation of RNA Stability and Protein Synthesis Using Thio-phosphate"
Examiner: Nancy T. Vogel
Art Unit:1636

Assistant Commissioner for Patents
Arlington, Virginia, 22313

RE: Response to Advisory Action Dated 8/15/08 and Non-compliant Notice as well as Oral communication on 8/26.

Sir,

An amendment was filed June 2, 2008 which failed to place application in condition for allowance. I am enclosing arguments that were faxed to the examiner after a phone conversation. These are attached to the statement of substance of the interview with examiner on 8/26/08. Below are some additional follow up comments after the phone interview for the examiner to consider. Since my fax communication I have modified the claims further in particular claim 9 (see below). Please consider the present claim amendments included. This should also address the non-compliant notice as well.

I wish to request an extension of time for two months for the delay this has caused. Enclosed are forms for an extension of time and as well as a transmittal form. I also wish to request a continued examination. RCE forms are enclosed as well.

I wish to make an amendment to the specification to now include Figure 3 of the parent application along with the description of the relevant experimental details. This figure was added to the parent application that issued and was not included as a part of the present application. This would assist with the present application as indicated by the examiner in the interview. This would not constitute new matter but simply make available material from the original parent application in the present application. Note the amended specification has been included and the sections to be added are underlined. I have also included the figure for figure 3.

Regarding Claim 9 USC 112 -Update since communication on 8/26/08

I wish to address the general applicability of claim 9. Phosphate is a nutrient that is universally needed by cells particularly to synthesize nucleic acids such as RNA and DNA. Thio-phosphate is an analog of phosphate that simply has one oxygen atom substituted with a sulfur atom. I have demonstrated that thio-phosphate can be utilized by a variety of organisms such as bacteria, yeast, and fish. In all instances thio-phosphate is incorporated into NTP pools and used in the production of nucleic acids. Note that deoxynucleotides are synthesized from ribonucleotide precursors via Ribonucleotide reductase. Examiner's reference Sun and Allfrey (1982) demonstrates the incorporation of radioactive thio-phosphate into AMP-s-alpha pools in Hela cells as well.

Experiments with fish (presented in original specification) actually demonstrate the uptake of thio-phosphate into a multi-cellular organism. An excess of thio-phosphate was needed to help with competition from endogenous sources of phosphate in the organism. Possible sources of phosphate in the organism could be the blood and bone if resorbed. Nevertheless entry into DNA as judged by the generation of DNase resistant genomic DNA,implies entry into RNA as well given that ribonucleotides are used to generated deoxynucleotides used for DNA synthesis. Incorporation into RNA as also been demonstrated for bacteria and yeast.

The present invention provides support and direction for the uptake and delivery of thio-phosphate into multi-cellular organisms. This is an extension of the body of data from all three related patent applications and is supported by direct experimentation. Given the universal need for phosphate claim 9 can reasonably be seen as speaking to any given complex organism.

Claim 9 is the only claim to address this aspect of the invention from the original patent application. Because of this, I have modified claim 9 to include both DNA and RNA. When the parent application was split into 3 patent applications this got lost in the shuffle as I tried to have the first patent application focus on DNA and the second on RNA.

That is also why Figure 3 was included in the patent that has issued and not in the present patent application.

Regarding Claim 10 USC 112 p. 1

An additional and more obvious argument for the present invention showing that the relative stability of mRNA is affected is as follows. I showed that protein expression of the bacterial AP gene in yeast could be enhanced 10 fold with only a 10% ratio of thio-phosphate to normal phosphate in the culture media. At the same time total protein synthesis in yeast did not increase 10 fold (see experimental section). Therefore the increase in protein synthesis of the AP gene is likely to have come at the expense of other proteins. I have argued based on the mechanism of action that less stable mRNA accumulate preferentially relative to more stable mRNA with the addition of thio-phosphate to the media. As the cell reaches its synthetic capacity for protein, the expression of very stable mRNA products is presumably reduced.

Comments on Original Amendments to the Specification

When the present application was prepared the specification was modified to include additional references up to the date of application that represented the status of the current state of the art. This was done to help distinguish the present invention from other works and these were included in the **description of related disclosures.** This did not constitute new matter and was mentioned when the application was submitted.

Two sections below where also added in the **description of preferred embodiments.** I wish the examiner to determine if they should be considered as new matter. If so these sections can be deleted or perhaps a continuation in part after an RCE is granted. The second paragraph is an extension of the descriptive section of the specification.

The ability to modify the atom adjacent to phosphate may be significant for in vivo NMR imaging; particularly since ^{31}P has such a high natural abundance and high magnetic moment (Lesney, M.S. (2003) Modern Drug Discovery 6:37-39). It might be possible to detect growing cells, dead cells, and/or resting cells using thio-phosphate as an imaging reagent.

Enhanced expression and yields of mRNA per cell may be quite useful for diagnostic applications. In particular, increased mRNA levels may be beneficial for human genetic tests where nonsense mediated decay of mutant genes and/or low level expression limits their detection in lymphocytes by RTPCR. In addition, it should be possible to culture pathogenic micro-organisms in the presence of thio-phosphate and look at gene specific markers of interest to assist in detecting and/or classifying the disease state. Furthermore, the ability to stimulate mRNA production may be useful for inducing cell differentiation in culture which may aide in the creation of artificial organ systems for the liver as well as other cultured products. In this regard it is not inconceivable that the compound could have therapeutic benefits for humans as well

I wish to declare and make a verified statement that the modified claims include no new matter as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825 (d).

Please under MPEP 707.07(j) the pro se applicant requests that if the Examiner fines patentable subject matter disclosed in this application, but feels that applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant

Respectfully submitted,

Elizabeth Frayne

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CLAIMS AMENDMENTS

What is claimed is:

Claims 1-7 (canceled)

8. (currently amended)

A method for stabilizing and/or accumulating cellular RNA in vivo resulting in an increase in the total amount of cellular mRNA per cell as well as the protection of RNA from degradation during subsequent isolation procedures ~~accumulation of rarer and particularly unstable mRNAs inside cells such as RNA processing intermediates~~ comprising:

1) preparing microbial culture media depleted of phosphate
2) adding thiophosphate as the sole source of phosphate to the media
3) culturing micro-organisms in the modified media for a sufficient time such that most mRNAs and/or rRNAs are modified with phosphorothioate linkages
4) ~~utilizing the stabilized RNA for easier detection owing to the enrichment of mRNA relative to rRNA in total RNA as well as the enrichment of unstable mRNAs relative to total mRNA and/or for the stabilization of particular RNA species during in vitro isolation and analysis via Northern blotting, RT-PCR, RNA protection assays, DNA chip assays and other similar RNA/RNA or other similar DNA/RNA based hybridization assays.~~

9. (currently amended)

A method for chemically modifying the RNA and/or DNA inside the cells of complex organisms comprising:

1) identifying the source of organic phosphate in the organism's diet
2) removing the organic phosphate source from the diet by ~~either~~ substituting thiophosphate for organic phosphate in the diet or alternatively adding an excess of thiophosphate to the diet such that it effectively competes with the normal phosphate present for incorporation into cellular nucleotide pools

3) feeding thio-phosphate to the organism by adding it to solid feed, or by feeding the organism a liquid source of thio-phosphate, or alternatively by injecting thio-phosphate directly into the organism.

10. (currently amended)

A method to ~~preferentially~~ enhance the synthesis of a ~~population of~~ proteins ~~and/~~or a group of ~~protein~~ proteins ~~of interest~~ by stabilizing ~~the~~ their corresponding mRNA(s) in vivo comprising:

1) growing cells in thio-phosphate containing media
2) determining the optimal ratio of thio-phosphate to inorganic phosphate in the culture media such that the desired mRNA(s) ~~specie(s) along with those mRNAs having similar half[[-]]lives are~~ is preferentially stabilized and translated inside cells ~~relative to naturally abundant and more stable mRNA species as evidenced by the enhanced protein expression of one or more gene products of interest~~
3) determining the optimal time point to induce stabilization of the desired mRNA(s) ~~of interest~~ via the addition of thio-phosphate containing media to growing cultures that results in maximal ~~for enhanced~~ protein synthesis ~~expression~~ of the corresponding gene product(s).

11. (previously amended)

The method of claim 10 wherein the cells used to generate the proteins or protein of interest are prokaryotic or eukaryotic in origin.

12. (previously amended)

The method of claim 10 wherein the proteins or protein of interest is produced using a recombinant DNA or viral expression vector or is a viral protein produced by an intact virus.

13. (currently amended)

The method of claim 10 wherein the enhancement of one or more proteins in cells results in an increase in a rate limiting enzyme(s) needed for the synthesis of a given fermentation product including by way of example ~~reaction(s)~~ ~~and~~ ~~as such is used to increase the rate or yield of non-protein products such as~~ antibiotics, chemical intermediates, organic acids, etc. ~~in fermentation reactions.~~

14. (cancelled)